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Efficacy of practical stump treatment in Latvia

Uvis Skola

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Efficacy of practical stump treatment in Latvia

Uvis Skola

Supervisor: Jonas Rönnberg, SLU, Southern Swedish Forest Research Centre
Assistant supervisor: Tālis Gaitnieks, Latvian State Forest Research Institute "Silava"
Examiner: Emma Holmström, SLU, Southern Swedish Forest Research Centre

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Swedish University of Agricultural Sciences
Faculty of Forest Sciences
Southern Swedish Forest Research Centre

Abstract

Root and butt rot caused by *Heterobasidion* spp. result in severe economic losses in Norway spruce forests of the northern hemisphere. Stump treatment using biological or chemical control agents are amongst the most effective solutions of limiting introduction of *Heterobasidion* spp. after tree felling. The aims of my thesis were to test the control efficacy of Rotstop[®], native Latvian *Phlebiopsis gigantea* strain and urea as a control agent against natural spore infection of *Heterobasidion* spp. on pre-commercial thinning stumps of Norway spruce and to develop the methodology to increase the efficacy of control agents.

Sample plots in three Norway spruce stands located in eastern part of Latvia were pre-commercially thinned. In search of improved efficacy of control agents, two methods of preventing primary infection by *Heterobasidion* spp. were compared. First method concerned practical stump treatment with a control agent while according to second method protective cover (wooden disc) was placed on half of the stumps in each sample plot after treatment was applied. After 14 weeks a total of 476 sample discs were obtained and later on analysed.

Results concerning presence and magnitude of infection were not influenced by sample plot. All treatments within a method showed lower infection rates than corresponding control stumps. 35 % of not covered control stumps were infected with a mean relative infection area of 3.4 %. Infection frequency for treated stumps ranged from 13-18 % with a mean relative infected area ranging from 0.9 to 1.1 %. Infection rates for covered control and urea-treated stumps were higher but for native Latvian *P. gigantea* and Rotstop[®]-treated stumps it was lower compared to their not covered counterparts. Control efficacy in terms of reduced relative area of infection was similar for all treatments when stumps were not covered. When stump surface was covered, Latvian *P. gigantea* and Rotstop[®] showed greater values than urea (96 %, 99 % and 73 % respectively).

Stump treatment using Rotstop[®], native Latvian *P. gigantea* strain or urea as a control agent should be considered since all treatments significantly reduce the primary infection rate of *Heterobasidion* spp.

Keywords: Norway spruce (*Picea abies*), *Heterobasidion*, *Phlebiopsis gigantea*, Rotstop, urea, pre-commercial thinning, covered stumps, control efficacy

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1. Introduction

Heterobasidion spp. is a root and butt rot causing pathogen that mainly concerns coniferous trees in northern hemisphere (Lind et al. 2014). Pathogen causes large economic losses as it both destructs living trees and affects stems by decreasing their quality and value (Bendz-Hellgren et al. 1999). Besides being directly detrimental to tree health, pathogen introduces an increased risk of windthrows (Rönnberg et al. 2006a). Roughly 23 % of mature Norway spruce (*Picea abies* (L.) Karst.) trees in Latvia are infected by *Heterobasidion* spp. (Arhipova et al. 2011; Kenigsvalde et al. 2016) indicating the gravity of this problem. Study by Gaitnieks et al. (2008) showed that the losses caused by *Heterobasidion* spp. in final fellings of Norway spruce stands in Latvia can range from 800 to 4790 EUR per hectare.

Primary spread of *Heterobasidion* spp. is caused by airborne basidiospores that infect stumps and tree wounds while secondary spread takes place afterwards, through root contact (Rishbeth 1951a; Rishbeth 1951b). Majority of wind-spread spores near the ground land within a 100 m range from fruiting bodies (Kallio 1970) while other studies show that a single spore can travel up to the distance of 500 km (Bérubé et al. 2017). Final felling and thinning of stands are infection gateways as both of the operations provide stumps and wounds that can get infected. Probability of primary infection by airborne *Heterobasidion* spp. spores, amongst other factors, is influenced by temperature sum (Mattila & Nuutinen 2007; Kasanen et al. 2011), humidity (Kallio 1970) and wood moisture (Redfern 1993). In Latvia these factors favour the sporulation of *Heterobasidion* spp. fruiting bodies the most from April till November (Brauners et al. 2014). Due to global tendency of increase in mean annual temperature, sporulation period of *Heterobasidion* fruiting bodies can be expected to increase as well leading to more severe damages (Trishkin et al. 2016).

Periods of high air temperature tend to result in high infection rates of *Heterobasidion* (Yde-Andersen 1962). Consequently, right timing for carrying out thinnings and final fellings can be a good measure for preventing or significantly decreasing the stump colonization by airborne spores of *Heterobasidion* spp. (Brandtberg et al. 1996). However, such measure cannot always be applicable in practical forestry, therefore other alternatives need to be concerned.

Stump treatment seems to be amongst the best solutions, when it comes to limiting the infection rate of *Heterobasidion* spp., as it is both an effective and for the most part profitable forestry action to carry out (Thor 2005; Thor et al. 2006). Common stump treatment practice is to apply chemical or biological control agent on freshly cut stumps surfaces. Amongst other chemical control agents, urea has been reported to be more favoured due to environmental concerns, effectiveness, price, availability and user-friendliness (Greig 1978; Brandtberg et al. 1996). Biological control agents in form of fungal species that compete with *Heterobasidion* spp. are an alternative for chemical stump treatment. Commonly used biological stump treatment in Europe is *Phlebiopsis gigantea* (Fr.) Jülich (Asiegbu et al. 2005) which is a saprophytic fungus that is capable of competing with *Heterobasidion* spp. (Holdenrieder and Greig 1988). Although *P. gigantea* is oftentimes naturally occurring on freshly cut stump surfaces, its infection rate is usually not high enough to stop *Heterobasidion* spp. of infecting stumps completely (Rönnberg et al. 2006b). *P. gigantea* is perceived as rather environmentally-friendly solution due to no proved negative effects on ground vegetation in contrast to urea (Westlund and Nohrsted 2000). Despite that, a negative effect on fungal species structure in freshly cut Norway spruce stumps has been reported (Vasiliauskas et al. 2004).

Commercially available product Rotstop® is a preparation that contains the suspension of oidia spores of *P. gigantea* obtained in Finland (Korhonen et al. 1994). Based on several previous studies, control efficacy of Rotstop® in Norway spruce stumps may range from 26 – 100 % (Berglund and Rönnberg 2004; Rönnberg et al. 2006a; Gunulf et al. 2012; Kenigvalde et al. 2016). Efficacy is influenced by many site specific factors but the rate of coverage of control agent has a great impact on the success of controlling primary infection by *Heterobasidion* spp. (Berglund and Rönnberg 2004).

Although Rotstop® has proved itself as an effective control agent against root and butt rot, several studies have reported native isolates of *P. gigantea* that are capable of achieving similar if not higher efficiency (Berglund et al. 2005; Rönnberg et al. 2006a; Kenigvalde et al. 2016). Since on-going stump treatment with a single genotype of *P. gigantea* could negatively impact native populations of this fungus (Vasiliauskas et al. 2004), having multiple isolates of *P. gigantea* available for market would be beneficial (Pratt et al. 2000). Consequently, testing the efficiency of different genotypes of *P. gigantea* and looking for ways to increase the efficiency would be beneficial for biocontrol of *Heterobasidion* spp.

The aims of this study were (i) to test the control efficiency of Rotstop®, native Latvian *Phlebiopsis. gigantea* strain and urea as a control agent against natural spore infection of *Heterobasidion* spp. on pre-commercial thinning stumps of Norway spruce, (ii) to develop the methodology to increase the efficiency of control agents.

2. Material and methods

Master thesis was developed in collaboration with Latvian State Forest Research Institute "Silava", department of Forest phytopathology and mycology.

2.1. Description of experimental stands

2.1.1. Criteria for stand selection

Stand selection for the study was based on several criteria. First of all, Norway spruce stands of similar age had to be growing on former agricultural land. Stands had to differ in site index or soil conditions to enable the ability of comparing treatment efficiency amongst different growth conditions. Previously unthinned stands had to be selected in order to prevent the possibility of having primary infection from airborne *Heterobasidion* spp. spores already present in experimental stands. Size of the stand and number of trees per stand were factors of interest as well, since 160 trees had to be cut down in each stand without removing fraction of basal area that would be detrimental to the health of these stands. In order to increase the chance of having successful experiment which would allow finding out the efficacy of treatments, there had to be a natural primary *Heterobasidion* spp. infection in experimental stands after thinnings were carried out. This probability was increased by checking for *Heterobasidion* spp. fruiting bodies in older Norway spruce stands that are bordering potential experimental stand. Fruiting bodies, if found, preferably were located near the border where stands met. Importance of sample plot distance from *Heterobasidion* spp. fruiting bodies can be stressed by the fact that majority of spores near the ground are deposited within a 100 m range from fruiting bodies (Kallio 1970).

2.1.2. General description of experimental stands

In total 3 stands located in the eastern part of Latvia (Figure 1) were used for obtaining field data within the context of the thesis. Stands have similar historical background regarding the type of previous land use, as all 3 of them were established on former agricultural land. Experimental stands share the same species and age but differ in other stand characteristics. Locations of experimental stands together with several other characteristics are shown in Table 1.

Table 1. Description of experimental stands

Experimental stand	Location (Lat./Long.)	Age	Area, ha	Forest type
1-8	56.24088, 27.88769	15	5.83	<i>Oxatidosa</i>
1-54	56.22804, 27.97499	15	2.38	<i>Oxalidosa turf. met.</i>
1-79	56.22430, 27.83745	15	8.44	<i>Hylocomisa</i>



Figure 1. Location of experimental stands

Forest types of experimental stands have been determined according to the forest typology of Latvia during previous inventories. Typology is based on site production, together with several ecological and biological characteristics (Avis 1997), thus it bears an impact on growth conditions present in the stands.



Figure 2. Experimental stand 1-8

Experimental stand 1-8 has been determined to fit forest type *Oxatidosa*. This type of forest establishes on sandy mineral soils where groundwater does not directly affect root system of trees. Slightly acid podzols and albeluvisols are the most common soils in forest type *Oxatidosa*. Site productivity up to $390 \text{ m}^3 \text{ ha}^{-1}$ for Norway spruce at the end of rotation period can be predicted for experimental stand 1-8 based on rather nutrient-rich soils with low groundwater level which are characterizing forest type *Oxatidosa* (Zālītis and Jansons 2013).



Figure 3. Experimental stand 1-54

Experimental stand 1-54 has been determined to fit forest type *Oxalidosa turf. met.* This type of forest establishes on wetlands with peat horizon or on fens when these areas are drained. Level of groundwater permanently decreases after drainage. Soils are nutrient-rich, medium acid, with thick peat horizon formed from well-decomposed sedges and tree remains. Based on the forest type, site productivity up to $400 \text{ m}^3 \text{ ha}^{-1}$ for Norway spruce at the end of rotation period can be predicted for experimental stand 1-54 (Zālītis and Jansons 2013).



Figure 4. Experimental stand 1-79

Experimental stand 1-79 has been determined to fit forest type *Hylocomisa*. Forest type establishes on sandy mineral soils where groundwater does not directly affect root system of trees. *Hylocomisa* is characterised by podzol soils with well-developed illuvial horizon. Norway spruce in forest type *Hylocomisa* has a good site productivity which is almost as high as it would be in forest type *Oxatidosa* (Zālītis and Jansons 2013).

2.2. Thinnings

Thinning operations, i.e. establishment of the experimental stands took place from 11.07.2018. to 17.07.2018. Taking into account the small diameters at breast-height that are present in all experimental stands, thinnings from above were performed using motor-manual labour. Workers were instructed to remove 160 Norway spruce trees in each experimental stand by carrying out thinnings with 45 % intensity based on number of trees per hectare. Intensity of thinnings was set according to pre-commercial thinning guides issued by joint stock company “Latvijas valsts meži”. Thinning guides are based on tree species, initial number of trees per hectare and mean height of trees and are applicable up to point where mean height of the stand is below 15 m. Instructions on thinning intensity were made on assumptions that experimental stands have not encountered pre-commercial thinnings before, there has not been strong self-thinning and that trees are planted with even spacing across the whole stand. However, these assumptions have proved to be not quite true. Experimental stands 1-8 and 1-79 have traces of light pre-commercial thinnings. Small diameter old stumps can be found at various places at these stands, but they are not distributed across the whole area of experimental stands. *Heterobasidion* spreads with a speed ranging from 7 to 12 cm per year in stump roots, although it can spread with twice the speed (Pettersson et al. 2003). Based on previously reported speed of secondary spread of *Heterobasidion* spp. a distance of 5 m was set as a buffer zone between experimental plots and old stumps. Such precautions were taken in order to avoid having already present *Heterobasidion* spp. secondary infection in experimental trees from stump roots remaining from previous pre-commercial thinnings.

Initial stump height of removed trees was 70 cm. Stumps were left at such height due to time of establishment, which happened almost one week after thinnings were carried out, and warm and dry weather conditions at the time of thinning. 70 cm high stumps gave the opportunity to saw off and waste the top 30 cm of stumps in order to imitate the conditions of freshly cut stump before application of treatment.

Initially, diameter threshold of 10 cm at the height of 40 cm, for trees to be removed, was set. Goal of having minimum diameter threshold was not fully met due to limited selection of larger dimension trees and poor execution of thinning instructions.

2.3. Establishment

2.3.1. Experimental design

Each experimental stand contains a single rectangular sample plot where thinning took place providing 160 stumps for the experiment. Treatments were assigned to stumps according to pre-prepared systematic scheme that was identical for all experimental stands. Stump treatment started from north-west corner of sample plot.

Experimental treatments (Table 2) were tested with two methods of treatment utilization as agent against primary infection by *Heterobasidion* spp. First method, not covered stump surface, describes stumps that were treated and then left as they were just as it would have been done in practice. Second method, covered stump surface, describes stumps that had wooden discs placed on top of them, covering the surface of stumps, after treatment was applied. This method of stump treatment was tested in search of improved efficacy of control agents. Second method is based upon the hypothesis that stump covers should prevent *Heterobasidion* spp. spores of landing on

stump surfaces thus limiting the probability of primary infection. Experimental design results in a total of 20 stumps per treatment per stand.

Table 2. Treatments

	Treatments			
Not covered stump surface	Control	<i>Phlebiopsis gigantea</i> 422	Rotstop®	Urea
Covered stump surface	Control	<i>Phlebiopsis gigantea</i> 422	Rotstop®	Urea

Control treatments represent stumps that were not treated against natural spore infection of *Heterobasidion* spp. therefore these stumps represent natural infection rate for experimental stands. *Phlebiopsis gigantea* 422 treatment is a solution made using native Latvian *P. gigantea* strain. Rotstop® is a widely used stump treatment containing Finnish strain of *P. gigantea*. Urea (carbamide) treatment is a chemical control agent against infection by spores of *Heterobasidion* spp. Urea was diluted in hot water resulting in 35 % urea solution that was used for the experiment.

2.3.2. Establishment of experiment

Experiment was established on 24th of July, 2018 on all three experimental stands. First step of establishment was removal of top 30 cm of high stumps left after thinnings resulting in 40 cm high stumps for applying treatment. Stumps, which were treated with one of the treatments requiring stump covering with a wood disc, were shortened down to 45 cm of height. Then, 5 cm thick discs were sawn off, to later serve as stump covers, again resulting in 40 cm high stumps for applying treatment. Prior the appliance of treatment, sawdust was removed from stump surface by blowing it off using chainsaws exhaust and pressing the throttle three times. This was done in order to imitate the conditions of practical stump treatment performed by harvesting head of a harvester.

Treatments were applied by evenly spraying solution across stump surface in such a manner where stump surface is fully covered by solution. Aim was to cover the surface with ~1 mm thick layer of solution. Average diameter of all stumps used in the experiment was 10.14 cm. Majority of stumps lied between 8 and 12 cm of diameter and received equal dose of treatment, although, some of the larger stumps required higher dose of solution to achieve full stump coverage and some of the smaller stumps received less solution. Average dose of treatment consisted of a total of 4000 µl of solution. Such dose was applied with five sprays, each of whom contained 800 µl of solution. Both Rotstop® and *P. gigantea* 422 were diluted to contain 5000 spores per ml meaning that a stump that was sprayed five times with either Rotstop® or *P. gigantea* 422 received 20000 spore treatment.

Stumps were marked by stapling plastic tags, containing stump ID, to their surface (Figure 5). Stumps, that represent treatments with wooden disc stump covers, were then covered with previously prepared protective discs with thickness of 5 cm.



Figure 5. Treated and marked stump

2.3.3. Meteorological conditions before and after establishment

Meteorological data for describing weather conditions of surrounding area before and after the establishment of experiment was obtained from State limited Liability Company's "Latvian Environment, Geology and Meteorology Centre" webpage. Data is provided by Rēzekne observation station and concerns hourly sum of precipitation, actual air temperature, wind direction and wind speed during a time period of six weeks (03.07.2018. – 14.08.2018.). Rēzekne observation station is located in the eastern part of Latvia, in a 50.68 km distance from experimental stand 1-8, 55.7 km distance from stand 1-54 and 49.82 km distance from stand 1-79, making it the closest observation station to stands used for the study.

During three week time period before the establishment of experiment actual air temperature was fluctuating from 8.9 to 28.3 °C with a mean of 19.1 °C. Sum of precipitation during this period was 27.4 mm. Higher temperature fluctuations was took place during following three weeks after the establishment of the experiment when actual air temperature was ranging from 8.9 to 30.5 °C with a mean of 20.3 °C. Sum of precipitation in following three week period after establishment was 51 mm. Rēzekne observation station reports a precipitation sum of 0.2 mm during time period from 24.07.2018. to 02.08.2018. when experiment was established. Rest of precipitation (50.08 mm) was recorded during following 12 days.

2.4. Sampling of discs

Sampling of sample plot located in experimental stand 1-54 took place on 31.10.2018. (14 weeks after establishment), wooden discs from stands 1-8 and 1-79 were sampled on 01.11.2018. Sampling of discs for laboratory analyses was carried out by sawing them off of stumps. With respect to practical stump treating, chainsaw bar was not sterilised prior sawing. Sawdust was removed from chainsaw in-between stumps by pushing the throttle. After cutting of 4-5 cm thick disc from the top of the stump and wasting it, 3 cm thick sample disc was cut off stump for laboratory analyses.

Sample discs were sawn all the way through their diameter until the bark of the opposite side and then tore off in order for them not to fly away and get contaminated. Each disc was stored in sealed plastic bag using plastic gloves, after marking its upper

surface with a cross for recognition purpose. Plastic bags were marked with disc's ID and the date of sampling and were kept sealed while transporting them.

Discs were transported to facilities of LSFRI "Silava", on the same dates when they were sampled, and stored in climate room with a constant temperature of 5 °C. Discs were taken out of the climate room, for incubation lasting 5-7 days, in such amount that incubated discs could be managed to analyse.

Prior incubation, discs were debarked and washed with a brush under running tap water in order to limit the possibility of unwanted contaminations. Discs were placed in a vertical position, after washing them, and left to dry. After discs had dried, each of them was stored in a separate polyethylene bag for incubation period. Bags were not sealed in order to maintain the circulation of air.

2.5. Disc analysis in laboratory

Disc analyses, where the lower surface of disc was examined, concerned all samples obtained from experimental stands and took place after an incubation period lasting 5-7 days. Discs were examined for conidiophores of *Heterobasidion* spp. and mycelium of *Phlebiopsis gigantea* at the same time. Stereomicroscope Leica MZ 7.5 was used for analysing discs.

In order to examine a disc for the area covered by conidiophores of *Heterobasidion* spp., a grid, consisting of 1 cm² large squares, was attached on the lower surface of a disc using pins. Squares, where *Heterobasidion* spp. conidiophores were present, were marked with a red dot on the disc's surface using waterproof marker (Figure 6).

Area covered by mycelium of *P. gigantea* was determined by its characteristic orange brown colouring and morphological features (mycelium is formed needle-shaped hyphae coated with calcium oxalate crystals) (Gaitnieks 2009). Areas where features were recognized were marked on disc surface using green waterproof marker. Area of discs surface, where mycelium of *P. gigantea* was not present, was marked with a green cross in order not to confuse both areas when measurements are carried out (Figure 6).



Figure 6. Disc containing conidiophores of *Heterobasidion* spp. (red dots) and mycelium of *Phlebiopsis gigantea* (green outline)

After disc analysing was finalised, areas of disc surface, containing conidiophores of *Heterobasidion* spp. and/or mycelium of *P. gigantea* together with discs circumference, were redrawn on A4 transparent plastic sheets (Figure 7). Circumference of discs and the area covered by mycelium of *P. gigantea* were measured in cm² using digital planimeter PLANIX 10S „Marble” by Tamaya.



Figure 7. Plastic sheet containing redrawn disc after analyses

2.6. Description of empirical material

Empirical material obtained from disc analyses in laboratory contains data of the total area of each disc (cm²) and the area that *Phlebiopsis gigantea* and/or *Heterobasidion* covers on each disc. Empirical material is sorted by experimental stand and type of treatment stump received. Four out of 480 stumps that the study concerns were not found, when experiment was sampled, therefore data is obtained from a total of 476 sample discs (Table 3). Three of stumps, that were not found, were situated in stand 1-8. Two of those received Rotstop[®] treatment and had their surfaces not covered, third one was a covered control stump. Another covered control stump was not found in stand 1-54.

Data was checked for normality using Shapiro-Wilk test with null hypothesis stating that data belongs to a normally distributed population. With alpha level of 0.05, test proved data concerning total area of discs, area of disc surface covered by *P. gigantea* and area of disc surface covered by *Heterobasidion* to be not normally distributed (p-values = 0.00021, <2.2e-16 and <2.2e-16 respectively).

Table 3. Number of sample discs obtained and analysed

Treatment	Method	
	Not Covered	Covered
Control	60	58
Phlebiopsis gigantea 422	60	60
Rotstop [®]	58	60
Urea	60	60

2.7. Calculations and statistics

Results regarding infection rate and control efficacy were calculated based on 2 types of data – whether infection by *Heterobasidion* spp. was found (yes/no) and area of stump surface that was infected (cm²). Data concerning presence of infection was calculated as proportion of infected stumps within site, method and treatment. Relative area colonized by *Heterobasidion* spp. was calculated for every disc as a fraction of infected wood from total surface area of sample disc. Both types of data were summarized obtaining means, standard errors and confidence intervals. Control efficacy, expressed as reduced proportion of stumps colonized by *Heterobasidion* spp. and reduced proportion of wood colonized by this pathogen, for each treatment was calculated according to following formula:

$$E(\%) = 100 - \left(100 * \frac{n_t}{n_u}\right), \quad (1)$$

where n_t represents proportion of colonized stumps or proportion of colonized wood for treated stumps and n_u represents proportion of colonized stumps or proportion of colonized wood for control stumps (Kenigvalde et al. 2016). Control efficacy was calculated within site, method and treatment. Since only three values per control agent were obtained, no further analyses were carried out regarding efficacy.

Influence of site, method and treatment on the presence of infection and relative infected area was examined with factor analyses in GLM (Generalized Linear Model) using Rstudio Desktop 1.1.456 software (© 2009-2018 Rstudio, Inc.). Data regarding presence of *Heterobasidion* spp. and relative area colonized by the pathogen were processed separately. No site-influenced significant differences were found therefore site as a blocking factor was removed from the models.

Relationship between method and treatment, concerning data of infection presence, was investigated with binomial distribution and logit as link in GLM. Further pairwise comparison of model's EMMs (Estimated Marginal Means) was carried out with 95 % confidence level in order to determine differences of *Heterobasidion* spp. presence amongst all treatments. Differences of pairwise comparisons were depicted with compact letter display. P-values adjustment according to Tukey method was used.

Relationship between method and treatment, concerning data of relative infected area, was investigated with Gaussian distribution and identity as link in GLM. Further pairwise comparison of model's EMMs was carried out with 95 % confidence level in order to determine differences of relative area occupied by *Heterobasidion* spp. amongst all treatments. Differences of pairwise comparisons were depicted with compact letter display. P-values adjustment according to Tukey method was used.

3. Results

3.1. Infection rate

Primary infection by airborne basidiospores of *Heterobasidion* spp. was found in all three experimental sites concerning all treatments to a certain extent. Experimental site did not show an influence on the results of infection rate ($p = 0.52$) therefore experimental site was not taken into account when depicting results.

Infection rate, in terms of proportion of stumps where infection by *Heterobasidion* spp. was recognized (Figure 8), turned out to be the highest for covered control stumps (70.7 %) while a noticeable share of not covered control stumps and covered urea-treated stumps were infected as well. 18.3 % of stumps that were treated with urea were infected when stump surface was not covered. Smallest proportions of stumps where infection was recognized can be found amongst stumps treated with *Phlebiopsis gigantea* 422 and Rotstop®. When stump surface was covered, these treatments did not surpass 5 % mark in terms of proportion of stumps that was colonized by *Heterobasidion*. When stump surface was not covered these treatments scored 13.3 and 13.8 % respectively.

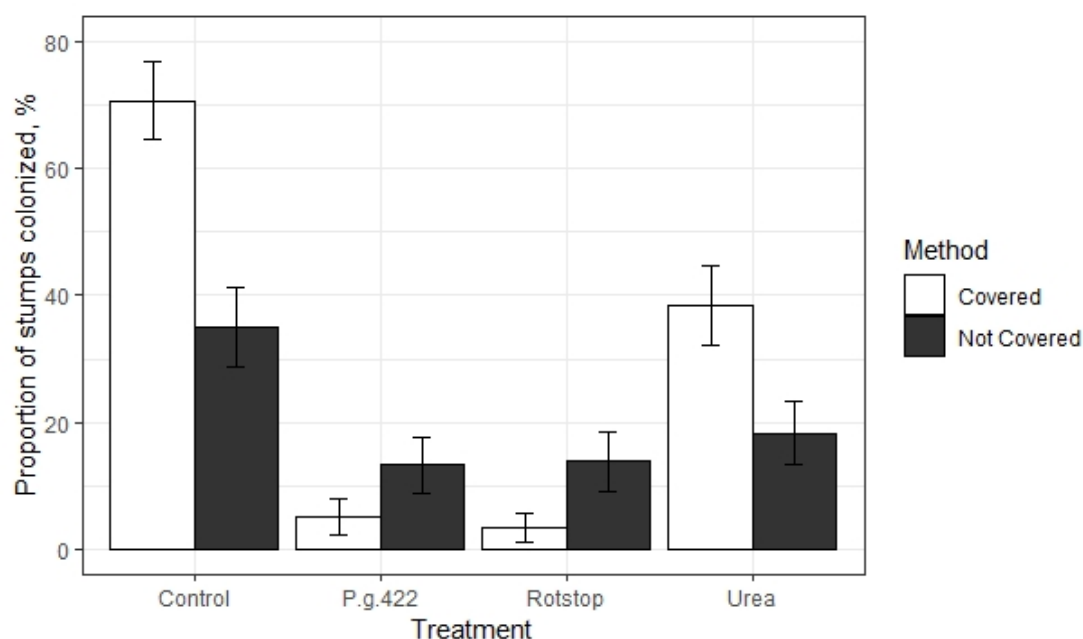


Figure 8. Proportion of stumps colonized by *Heterobasidion* spp. Bars show standard error for all samples within all sites.

Two factors that influenced these results are method ($p = 0.025$) and treatment ($p < 2.2e-16$). Covered control treatment had significantly higher infection by *Heterobasidion* spp. than any other treatment ($p < 0.05$). Not covered *P. gigantea* 422, Rotstop® and urea showed very similar results and also did not significantly differ from covered *P. gigantea*, Rotstop®, urea and not covered control ($p > 0.05$). However, not covered control stumps and covered urea treated stumps were significantly more susceptible than covered Rotstop® and *P. gigantea* 422 treated stumps ($p < 0.05$).

Infection rate depicted in Figure 9 show proportions of total colonized disc area from total disc area per treatment. Area occupied by *Heterobasidion* spp. between different treatments shows similar tendencies as the proportion of stumps colonized

by the pathogen. There was a trend to observe that mean area colonized by *Heterobasidion* spp. in covered control stumps was higher than in other treatments. Covered control stumps had the highest infection rate (10.18 %), followed by not covered control stumps (2.72 %) and covered urea treated stumps (2.37 %).

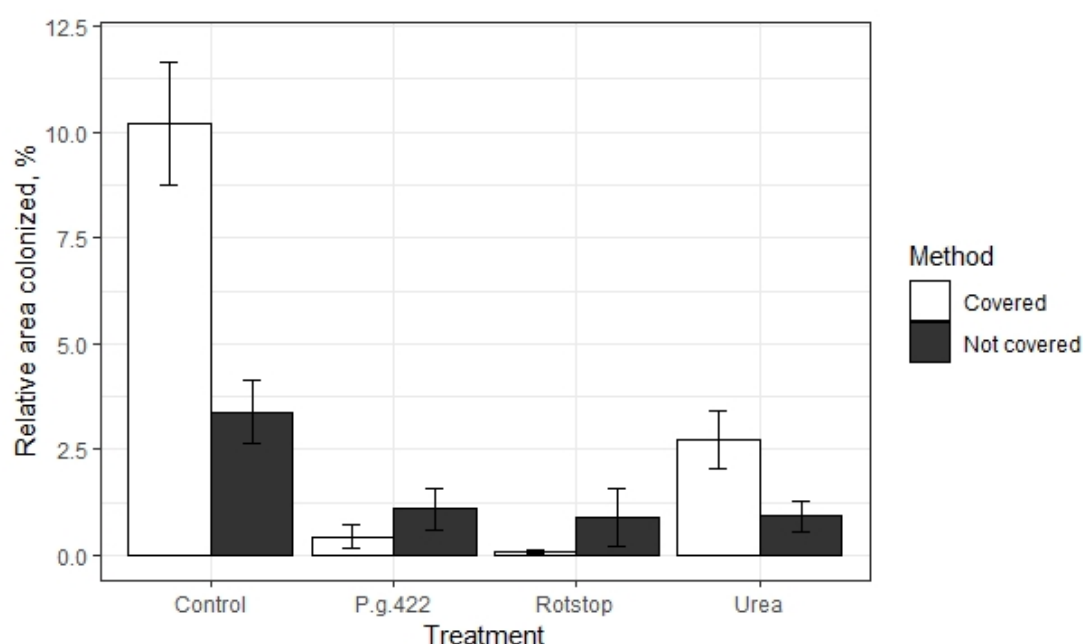


Figure 9. Relative stump area colonized by *Heterobasidion* spp. Bars show standard error for all samples within all sites.

Results shown in Figure 9 are influenced by both method ($p = 0.0112$) and treatment ($p < 2.2e-16$). Covered control treatment showed significantly higher infection by *Heterobasidion* spp. than any other treatment ($p < 0.05$). Similar area colonized by *Heterobasidion* spp. was observed in all treated stumps except covered urea treated stumps that were significantly more susceptible to infection by *Heterobasidion* spp. Covered urea treated stumps also showed similar results with not covered urea treated stumps and not covered control stumps ($p > 0.05$). *P. gigantea* 422 and Rotstop® treated stumps were significantly less infected than control stumps ($p < 0.05$). No clear trend on the influence of stump covering can be detected.

Table 4 shows the values and standard deviations of mean relative area infected by *Heterobasidion* spp. and proportion of infected stumps per site, method and treatment.

Table 4. Infection rate

Site	Method	Treatment	Stumps, n	Proportion of stumps infected, %		Mean relative area infected, %	
				Value	SD	Value	SD
1-8	Not covered	Control	20	45.00	51.04	5.18	7.08
		P.g.422	20	15.00	36.63	0.56	1.39
		Rotstop	18	16.67	38.35	0.28	0.79
		Urea	20	25.00	44.43	0.83	2.89
	Covered	Control	19	78.95	41.89	11.78	11.99
		P.g.422	20	0.00	0.00	0.00	0.00
		Rotstop	20	0.00	0.00	0.00	0.00
		Urea	20	30.00	47.02	1.86	4.65

1-54	Not covered	Control	20	25.00	44.43	1.81	4.71
		P.g.422	20	20.00	41.04	2.55	6.13
		Rotstop	20	25.00	44.43	2.33	8.76
		Urea	20	15.00	36.63	0.86	2.27
	Covered	Control	19	84.21	37.46	9.96	10.29
		P.g.422	20	0.00	0.00	0.00	0.00
		Rotstop	20	0.00	0.00	0.00	0.00
		Urea	20	40.00	50.26	1.94	4.38
1-79	Not covered	Control	20	35.00	48.94	3.18	5.38
		P.g.422	20	5.00	22.36	0.16	0.71
		Rotstop	20	0.00	0.00	0.00	0.00
		Urea	20	15.00	36.63	1.08	3.11
	Covered	Control	20	50.00	51.30	8.97	11.03
		P.g.422	20	15.00	36.63	1.32	3.61
		Rotstop	20	10.00	30.78	0.18	0.57
		Urea	20	45.00	51.04	4.41	6.33

3.2. Control efficacy

Control efficacy, in terms of reduced proportion of stumps colonized by *Heterobasidion* spp. and reduced relative area colonized by the pathogen compared to control stumps, is shown in Figures 10 and 11.

When calculated based on proportion of colonized stumps (Figure 10), control efficacy was the highest for *P. gigantea* 422 and Rotstop®. These treatments showed efficacy of 62 % and 61% when stump surface was not covered and 93 % and 95 % control efficacy when stump surface was covered. Urea treated stumps showed slightly higher efficacy when stumps surface was not covered with protective disc (48 % >46 %).

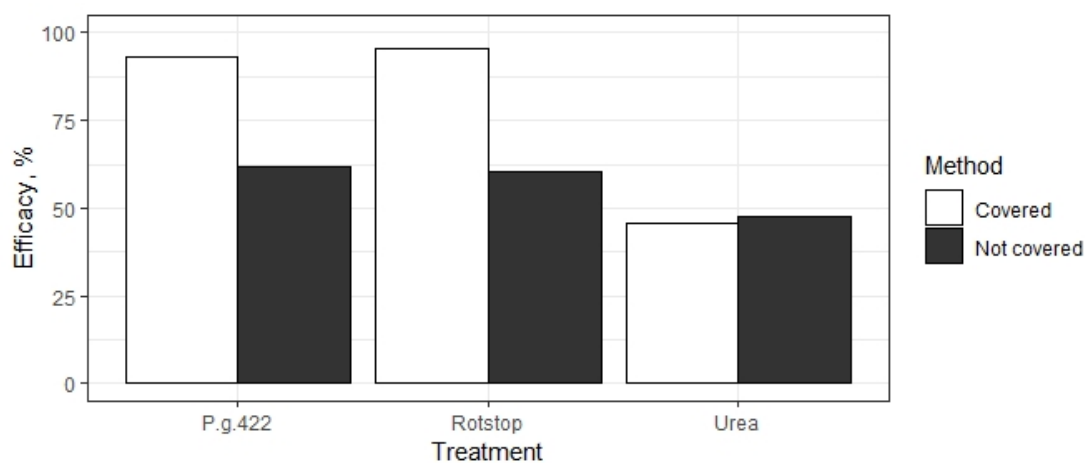


Figure 10. Control efficacy against *Heterobasidion* spp. based on proportion of infected number of stumps

Control efficacy for covered *P. gigantea* 422 (96 %) and Rotstop® (99 %) treated stumps was still the highest when calculated based on relative area of colonized wood (Figure 11). When not covered, Rotstop® treated stumps had the third highest efficacy (74 %) but stumps treated with *P. gigantea* 422 showed the lowest control efficacy (67 %). While urea showed very similar results between both methods, efficacy was slightly higher when stumps were covered after applying the treatment (73 % > 72 %).

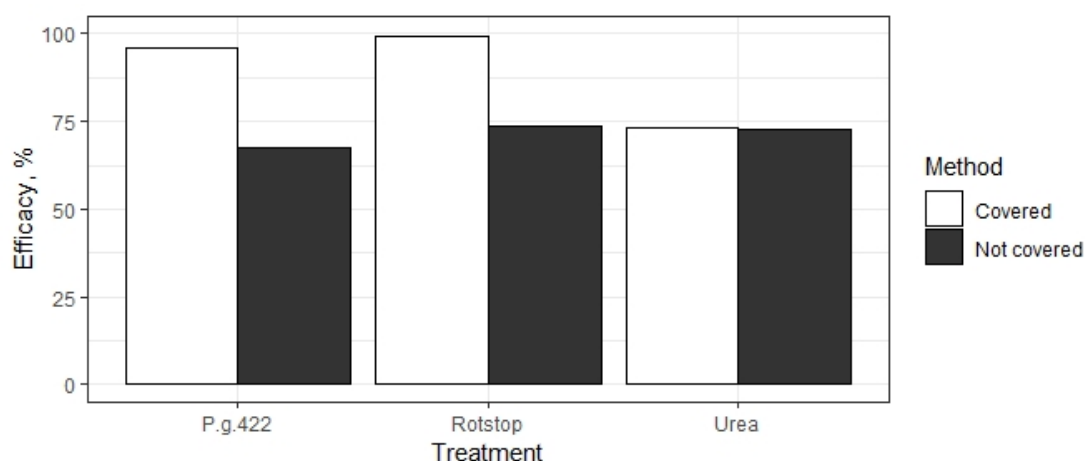


Figure 11. Control efficacy against *Heterobasidion* spp. based on relative area of colonized wood

3.3. Other results

Growth comparison of *P. gigantea* and *Heterobasidion* spp. is shown in Figure 12. Mean relative area occupied by *P. gigantea* was greater than the area occupied by *Heterobasidion* spp. not only for stumps that were sprayed with this fungus but for control stumps and stumps treated with urea as well. Both fungi showed statistically similar results for covered control stumps and for not covered stumps that received urea treatment ($p > 0.05$). There was more *P. gigantea* than *Heterobasidion* spp. in not covered control stumps and covered urea treated stumps ($p < 0.05$). Area covered by *P. gigantea* was the highest in *P. gigantea* 422 and Rotstop® treatments for both methods. Significantly more *P. gigantea* was found in *P. gigantea* 422 and Rotstop® treatments when stumps were covered ($p < 0.05$).

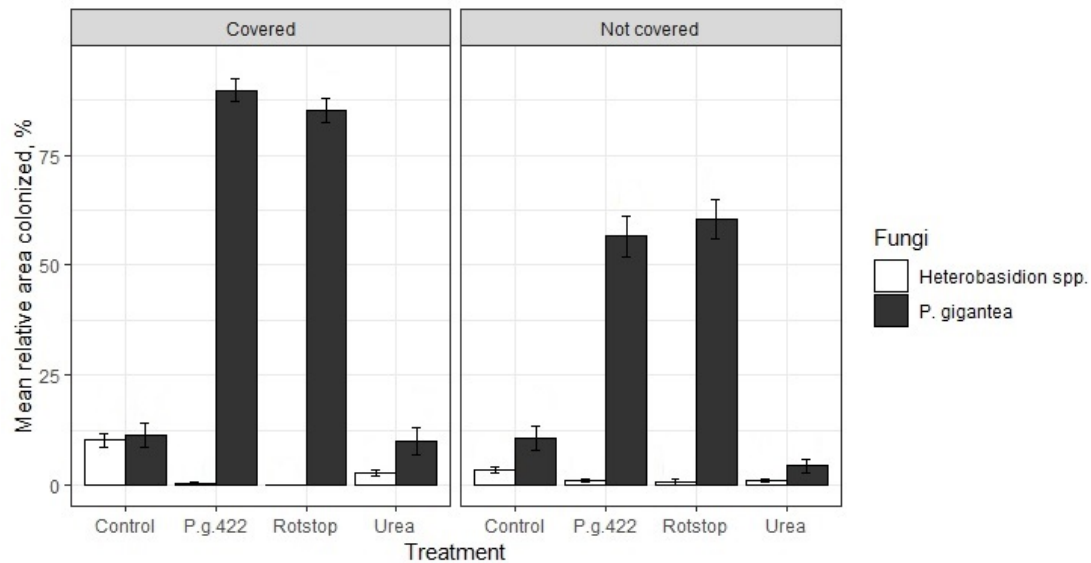


Figure 12. Mean relative area colonized by *P. gigantea* and *Heterobasidion* spp. Bars show standard error for all samples within all sites.

Natural dynamics of *P. gigantea* and *Heterobasidion* spp. in untreated control stumps are depicted in Figure 13. Figure concerns only those control stumps where both fungi were recognized. When stump surface was not covered, *P. gigantea* and *Heterobasidion* spp. both were present in a total of 9 stumps. When surfaces of control stumps were covered, a total of 19 stumps had natural occurrence of both fungi. Untreated stumps were analysed separately, revealing that the presence of naturally occurring *P. gigantea* had no influence on natural infection rate of *Heterobasidion* spp. ($p = 0.739$) but there were significant difference depending on the method ($p < 0.05$).

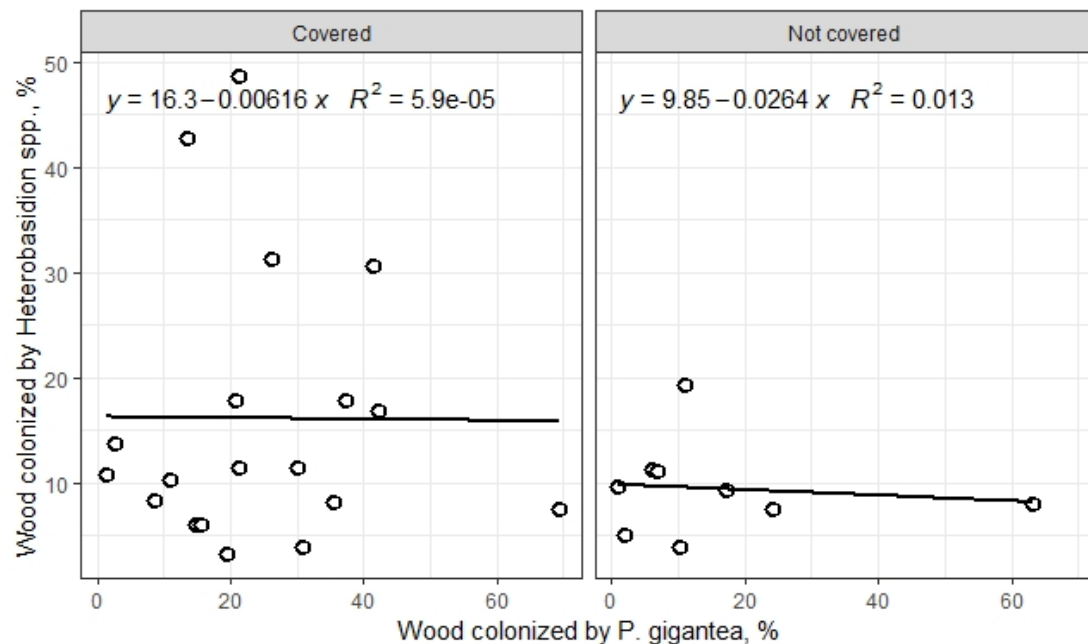


Figure 13. Relationship between relative area colonized by *Heterobasidion* spp. and relative area colonized by *P. gigantea* in control stumps

4. Discussion

Norway spruce stands are at risk of natural spore infection by *Heterobasidion* spp. during pre-commercial thinnings (Gunulf et al. 2012; Gaitnieks et al. 2018). My thesis supports previous findings as infection was found in all three pre-commercially thinned stands. Roughly one third of not covered control stumps were infected by *Heterobasidion* spp. Diameters of stumps, where *Heterobasidion* spp. infection was found, ranged from 5.5 to 13.6 cm for not covered control stumps and from 4.4 to 14.1 cm for covered control stumps. Gunulf Åberg et al. (2016) discuss the risk of transferring spores of *Heterobasidion* spp. from bark onto the stump surface while cutting it with chainsaw. They found that spores can indeed be transferred causing increase in the area infected by *Heterobasidion*. Removal of bark or sterilization of chainsaw bar between cuts is advised (Gunulf Åberg et al. 2016) but, since my thesis focuses on practical approach of stump treatment, such measures were not taken. Stump treatment using Rotstop[®], urea or native Latvian *P. gigantea* is an effective measure to be taken in order to prevent primary infection by *Heterobasidion* spp. since treatments significantly decrease the infection rate for both methods ($p < 0.05$).

Infection rate and control efficacy against *Heterobasidion* spp. were estimated using two different methods – proportion of infected stumps and relative stump surface area. Several previous studies that have used these methods of calculations, reveal that there are differences in results between both methods (Thor and Stenlid 2005; Berglund and Rönnerberg 2004; Kenigšvalde et al. 2016). In my thesis, results regarding infection rate show generally similar relationship amongst treatments with both methods of calculation but using relative stump surface area infected by *Heterobasidion* spp. infection rate values are lower but efficacy values are higher. This means that, while large proportion of stumps was infected, infections were small.

Efficacy varies more when results from both calculation methods are compared. When stump surface is not covered, Rotstop[®] and *P. gigantea* 422 have higher efficacy than urea, if proportion of colonized stumps is used for calculations. Calculations that are based on relative colonized stump surface area show lower value for *P. gigantea* 422 when compared to urea treatment ($67.33 \% < 72.71 \%$). Second method of calculating efficacy generally results in higher values (Kenigšvalde et al. 2016), results from my thesis support this claim as all treatments scored higher values when efficacy was calculated based on relative colonized wood area.

Success of biological control using *P. gigantea* depends on coverage of solution. Berglund and Rönnerberg (2004) reported that such control agents perform the best when stump surface is fully covered. Stumps in my experiment received full coverage. In practice, full coverage, when stumps are treated mechanically, is not always possible unless excess amount of solution is used (Pratt and Thor 2001).

Temperature is one of many factors that can influence the growth of *Heterobasidion*, therefore the results of an experiment. Previous study by Gooding et al. (1966) shows a decrease in growth of *Heterobasidion annosum* in stem wood when temperature exceeds 30 °C. Rēzekne weather observation station shows records of only three hours, during three week period after my experiment was established, when actual air temperature exceeded 30 °C. Based on meteorological data, there is no reason to suspect unusual growth of *Heterobasidion* in my experiment.

Half of stumps used for the experiment were covered with protective wooden discs that were cut from the same stump. Stump covering using plastic sheets or bags is occasionally done in order to protect treated stumps from rain (Redfern 1993; Morrison and Redfern 1994; Redfern et al. 1997; Tubby et al. 2008). In such cases

protective cover is removed after 24 hours. However, just as in this experiment, stump covering is used for increasing efficacy of biological control agents as well. Study by Varese et al. (2003) notes that stump covers protect the stumps from solar radiation and drying out. Moss and soil have been used as protective covers for stumps in previous studies (Cech et al. 2008; Volchenkova et al. 2013). Although results from these studies vary amongst experimental sites, authors conclude that stump covers are beneficial for the growth of *P. gigantea*. This statement falls in line with findings of Redfern (1982) who reported that covering stumps with freshly cut branches decreases variation in microclimate and supports the development of various fungal fruiting bodies. Results from my thesis further supports these findings as more *P. gigantea* and *Heterobasidion* spp. were found in covered stump treatments. Increased formation of *Heterobasidion* spp. fruiting bodies on covered Norway spruce stumps has been reported by Paludan (1966). Latvian State Forest Research Institute "Silava" have observed (unpublished data) that fruiting bodies of *Heterobasidion* spp. on Norway spruce stumps are more common when stumps are covered with moss, needles or twigs.

Redfern (1993) found out that covered Sitka spruce (*Picea sitchensis* (Bong.) Carr.) stumps (stumps were covered with polyethylene sheet ~60 cm above their surface) tend to get more infected by *Heterobasidion* spp. spores compared to not covered stumps. While stumps in my experiment were covered with a different method (stump covers were placed directly on stump surface), similar results were found. Covered control stumps had significantly higher *Heterobasidion* spp. infection than not covered control stumps ($p < 0.05$). In order to find the reason behind these strange results further research is needed but it might be that the spores were able to land under stump cover resulting in failed protection and furthermore creating suitable environment for pathogen's development. Study from Italy showed another chemical control agent (propiconazole) which performed worse when stumps were covered (Nicolotti et al. 1999).

All control agents had similar performance in mitigating the stump surface area covered by *Heterobasidion* spp. except covered urea treated stumps that performed worse. Stumps that were treated with native Latvian *P. gigantea*, Rotstop® or urea were significantly less susceptible against primary infection by *Heterobasidion* spp. when compared with untreated control stumps ($p < 0.05$). This means that the use of these control agents would be beneficial to the health Norway spruce stands. Rotstop® and urea are commercially available products but since urea is known for having negative effects on ground vegetation (Westlund and Nohrsted 2000), biological control using Rotstop® seems like the most appropriate approach of preventing the primary infection of *Heterobasidion* spp. in spruce stands of Latvia. On the other hand, Vasiliauskas et al. (2004) notes that on-going stump treatment with a single genotype of *P. gigantea* could negatively impact native populations of this fungus. Since native Latvian *P. gigantea* was similar with Rotstop® in terms of mitigating the infection by *Heterobasidion* spp., stump treatment using this and preferably other native strains of *P. gigantea* should be considered in the future.

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